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(12) **Unexamined Patent Application**

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(54) Process for the stabilization of infusion solutions that contain amino acids

Up to this point in time, infusion solutions, which contain amino acids, have been stabilized against oxidative decomposition via gassing with an inert gas, or by adding sulfite. Oxygen cannot be removed completely by gassing with an inert gas during manufacture. Sulfite reaction products are being correlated increasingly with serious incidents during infusion therapy.

As a result of the addition of less than 0.5% of cysteine, N-acetylcysteine or their salts and esters as a stabilizer, decomposition reactions, which are caused by residual oxygen or oxygen that has diffused [inward] during storage, can be avoided.

Stabilization of infusion solutions, which contain amino acids, against oxidative decomposition.

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Specification

The invention pertains to the stabilization of infusion solutions, which contain amino acids, against oxidative decomposition in accordance with the above claim. Such solutions are necessary for parenterally feeding persons who absolutely cannot, or cannot adequately, be fed orally. They [these solutions] have to be sterile, pyrogen-free, low in particulate matter, and chemically stable.

They can easily be impaired in regard to their chemical stability by temperature stressing during sterilization, as well as by exposure to light (1). Tryptophan (2-4), tyrosine, histidine, and methionine (4), in particular, have proven to be especially sensitive to oxidation. Maillard reactions can then take place between the oxidation products of these substances and the intact amino acids, whereby these cause concentration losses and the formation of agglomerates and, possibly, mutagenically acting reaction products (5,6).

These processes are recognizable by relatively simple means via the increasing yellow coloration of the solutions, together with an increase in extinction in the longer wavelength UV region (> 300 nm).

Since the problem of oxidative decomposition is known, two processes, in particular, have been used for stabilization purposes. On the one hand, oxygen is largely removed from the solution and the empty bottles by gassing with the inert gas nitrogen, along with evacuation of the dead space prior to placing the rubber stopper thereon. Under favorable circumstances, losses of significantly less than 10% of the saturation concentration are achieved in this case. Complete removal of the oxygen does not appear to have been possible so far. In addition, the vacuum in the bottle brings about a noteworthy increase in oxygen content during storage.

In addition, sulfite is occasionally added to such solutions in order to bind oxygen, especially when cysteine is contained therein as an active component. However, the sulfite reaction products are increasingly being correlated with serious incidents during infusion therapy, such as anaphylactic shock, asthma attacks, nausea, and diarrhea, whereby this has already led, as a consequence, to a corresponding announcement by the Federal Health Office (7).

The task, which forms the basis of the invention, is to prevent - by means of physiologically innocuous ancillary substances - oxidative reactions in infusion solutions, which contain amino acids, beyond the extent that is achievable using the possibilities of gassing with an inert gas.

This can be achieved by means of an addition of cysteine or N-acetylcysteine. The substance is an essential amino acid only in the case of those who are born prematurely, whereby the artificial supply thereof is not required in any other cases even with long-term parenteral feeding. The main oxidation product, cystine, is also physiological [sic]. The efficiency of the compound has already been proven, for example, in stabilizing epinephrine solutions that are significantly more unstable still (8).

As a result of the addition of these substances, it is possible to reduce the degradation reactions that are caused by residual oxygen and oxygen that diffuses inward during storage, especially in the case of exposure to light, in a clearly physiologically tolerable manner.

Usage Example

A 10% amino acid solution with the composition in accordance with Attachment 1 was mixed with different concentrations of N-acetylcysteine. The effects of sterilization and exposure to daylight were examined with the help of the test methods that are described in Attachment 2.

As can be seen from Table 1, it was not possible to establish any unambiguous difference in regard to the effect of sterilization. However, a slightly better result can be registered in the case of the preparation that contains acetylcysteine.

Table 1

Measured Values Before and After Sterilization

Formulation Time of Testing	1 Before sterilization	1 After sterilization	3 Before sterilization	3 After sterilization
O_2 % Saturation	8.5	7.6	8.7	7.3
$E\lambda 325$	0.028	0.032	0.028	0.025
Color	<G 9	<G9	<G9	<G9

Table 2

MEASURED VALUES AFTER DAYLIGHT EXPOSURE AT A WINDOW BETWEEN 03.10.88 AND 04.14.88

Formulation	1	2	3	4	1	2	4
Testing after Days	14	14	14	14	35	35	35
O_2 % saturation	2.5	2.4	1.8	1.7	1.8	1.2	0.8
$E\lambda$ 325	0.242	0.047	0.047	0.045	0.196	0.052	0.049
Color	BG 7	G 9	G 9	G 9	BG 6+	G 9	G 90

$E\lambda$ 325: Extinction at a wavelength of 325 nm; 10 mm layer thickness;
Color: Specification in accordance with the color tables in the German Pharmacopeia, 9th edition;
BG = brown-yellow, G = yellow.

However, pronounced differences are found if the solutions are subsequently exposed to daylight for only 14 days. Whereas only an extremely slight increase in color occurs in the case of the solutions with N-acetylcysteine, the coloration changes significantly, by more than two levels, in the case of the preparation that is free from ancillary substances, and the extinction at 325 nm is increased distinctly as well.

Practically no further change occurs after a further 21 days in the case of formulations 2 and 4. The color of the starting solution, by contrast, becomes more intense by more than one color level. The extinction also increases again.

The oxygen content decreases markedly in the case of all the preparations, whereby this is a consequence of oxidation reactions.

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Attachment 1

Composition and Preparation of the Model Solution

1. Composition

1,000 ml contain:

L-isoleucine	5.0 g
L-leucine	7.4 g
L-lysine acetate	9.31 g
L-methionine	4.3 g
L-phenylalanine	5.1 g
L-threonine	4.4 g
L-tryptophan	2.0 g
L-valine	6.2 g
L-arginine	12.0 g
L-histidine	3.0 g
Aminoacetic acid	14.0 g
L-alanine	15.0 g
L-proline	15.0 g
Acetic acid	8.01 g

Formulation 1: unchanged
Formulation 2: addition of 0.4 g/l of N-acetylcysteine
Formulation 3: addition of 1.0 g/l of N-acetylcysteine
Formulation 4: addition of 2.0 g/l of N-acetylcysteine

2. Preparation

- dissolution of the substances in distilled water for injection purposes at 55°C
- complete expulsion of the oxygen by means of nitrogen and cooling to 30°C
- filtration over filter elements with a pore width of 0.2 µm
- tapping off into oxygen-free infusion bottles: DIN light 500 ml
- sealing of the bottles with bromobutyl rubber stoppers and pull-off flanged caps
- sterilization with a modified hot water irrigation process at 121°C/15 minutes

Attachment 2

Data Regarding the Measurement [typo] Methodology

Apparatus: Oxo Digi 550 from the WTW firm in Weilheim
Measurement principle: Clark electrode
Evaluation of the measured values: conversion of the measured values, which were obtained in mg/l, into % of the saturation concentration at the temperature in question
Calibration of the apparatus: by determining the zero point in 3% sodium sulfite solution on each occasion, and measuring the oxygen concentration of air-saturated water in accordance with the operating instructions

2. Measurement of the extinction

Apparatus: recording spectrophotometer from the Bausch & Lomb firm with quartz cells and a 10.00 mm layer thickness
Measurement principle: by recording the spectra of solutions, which have decomposed to different degrees of intensity, it was possible to demonstrate that measurements at a wavelength of 325 nm permit accurate differentiation

3. Determination of the degree of discoloration

The indicated parameters pertain to the color dilutions as described in German Pharmacopeia, 9th edition. Since the yellow and the brown-yellow dilution series end at level 6, gradations 7-9 were prepared in accordance with the procedure that is indicated for the brown series.

Patent Claim

Process for the stabilization of infusion solutions, which contain amino acids, against oxidative decomposition, **characterized by the feature** that, as a stabilizer, 0-0.5% of cysteine or N-acetylcysteine, or their salts and esters, is added to the solution.